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INTRACELLULAR FATE OF PHASE I COXIELLA BURNETII IN GUINEA PIG P--ETC(U)
DEC 80 J S LITTLE, R A KISHIMOTO

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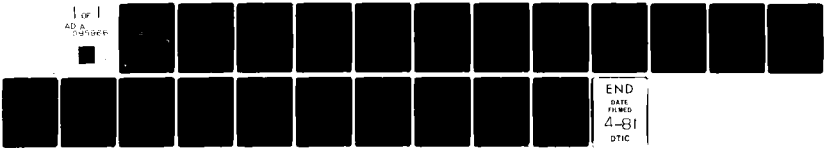

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Guinea pig peritoneal macrophages were infected with phase I Coxiella burnetii to assess the intracellular distribution of ingested rickettsiae. Localization of organisms was determined by fractionation of macrophage homogenates by equilibrium density centrifugation on sucrose gradients. Results show that in the absence of immune serum, ingested C. burnetii are not sequestered within macrophage lysosomes. Sequestration and subsequent degradation of rickettsiae within lysosomes occurs only when C. burnetii are opsonized with immune serum.

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Intracellular Fate of Phase I Coxiella Burnetii in Guinea Pig Peritoneal Macrophages

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Running Title: C. BURNETII IN PERITONEAL MACROPHAGES

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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ABSTRACT

Cultivated guinea pig peritoneal macrophages were infected with radiolabeled phase I Coxiella burnetii, in order to assess the intracellular distribution of ingested rickettsiae. Localization of organisms was determined by fractionation of macrophage homogenates by equilibrium density centrifugation on sucrose gradients. Macrophages isolated from either nonimmune or immune guinea pigs and infected with C. burnetii opsonized with immune serum yielded equilibrium density distributions for rickettsiae similar to lysosomal enzymes, suggesting sequestration within macrophage lysosomes. To confirm these observations nonimmune or immune guinea pigs were injected with Triton WR-1339 prior to macrophage harvest to decrease the density of macrophage lysosomes. Triton-laden macrophages infected with opsonized rickettsiae resulted in equilibrium density distributions for lysosomal enzymes and organisms in less dense regions of the gradient. In contrast, when either nonimmune or immune macrophages were infected in the presence of normal guinea pig serum, the distribution of labeled rickettsiae in the gradient did not correspond with lysosomes. We conclude that in the absence of immune serum, ingested C. burnetii are not sequestered within macrophage lysosomes. Phagolysosomal fusion and subsequent degradation of rickettsiae within the lysosomes of the macrophages appear to occur only when C. burnetii are opsonized with immune serum.

Coxiella burnetii is an obligate intracellular parasite which causes Q fever in man and a number of other animals (for review see ref. 17). The pathogenesis and the mechanisms of immunity of this disease are not well understood. Two host-controlled variants of C. burnetii designated phase I and phase II have been described (29). In nature or in laboratory animals C. burnetii exists in the phase I state. Repeated passage of phase I organisms in embryonated chicken eggs results in their gradual conversion to phase II. In vitro studies have shown that phase I and phase II rickettsiae have different viabilities in guinea pig peritoneal macrophages depending on both the immunological status of the animal from which the macrophages are harvested and on whether nonimmune (normal) or immune serum is used in the assay (19, 20). For the experiments described here, only phase I organisms were employed.

Previous in vitro studies have shown that phase I C. burnetii which had been pretreated with either nonimmune or immune serum was phagocytized by macrophages harvested from both nonimmune and immune guinea pigs (20). However, after ingestion, phase I C. burnetii which had been pretreated with nonimmune serum multiplied in and destroyed both nonimmune and immune macrophages (20). In contrast, when phase I C. burnetii were pretreated with immune serum, they were destroyed by both nonimmune and immune macrophages (11, 18-20). Although immune serum does not appear to have a direct rickettsiacidal action on C. burnettii (1) or other rickettsiae (7, 13), it has been shown to modify infection when passively transferred to nonimmune animals (1). The mechanism by which immune serum modifies infection when passively transferred to nonimmune animals or potentiates the destruction of phase I C. burnetii in both nonimmune and immune macrophages is not known. The experiments

described here were initiated in an attempt to provide more insight on the mechanism by which immune serum potentiates the intracellular destruction of phase I C. burnetii in guinea pig peritoneal macrophages.

MATERIALS AND METHODS

The chemicals, animals, culture and radiolabeling of rickettsial stock suspension, preparation of guinea pig peritoneal macrophages, infection of macrophage cultures with radiolabeled rickettsiae, UV light irradiation of infected macrophage cultures, homogenization of guinea pig peritoneal macrophages, fractionation of macrophage cytoplasmic extracts on linear sucrose gradients, determination of enzyme and radioactivity, and presentation of results were all as previously described (23). For a schematic review of the experimental protocol, see Fig. 1.

In addition, for experiments utilizing macrophages from immune animals, macrophages were harvested from guinea pigs which were immunized intraperitoneally (i.p.) at 4-week intervals with 0.2 ml of an organism suspension containing approximately 10^9 formalin-killed phase II C. burnetii.

Finally, for experiments where macrophage lysosomes were preloaded with Triton WR-1339 (Ryger Chemical Co., Irvington, N.Y.), peritoneal exudate cells were collected 4 days after guinea pigs were injected i.p. with 20 ml of a solution containing 1.5% (w/v) sodium caseinate and 2.5% (v/v) Triton WR-1339 in normal saline.

RESULTS

Fig. 2A shows an equilibrium density profile of the macrophage postnuclear supernatant obtained from immune macrophages which had been infected with radiolabeled phase I C. burnetii. For simplicity, only the profiles of two lysosomal marker enzymes and radioactivity are shown. Profiles of radiolabeled phase I C. burnetii and other macrophage organelle marker enzymes were shown previously (23). As shown in Fig. 2A, the radioactivity (solid line) representing the C. burnetii and the lysosomal marker enzymes peaked in the same fractions of the gradient, suggesting that under these conditions C. burnetii were associated with the lysosomes. However, phase I C. burnetii and lysosomes have similiar densities in alkaline sucrose (23). Hence, it was necessary to alter experimentally the equilibrium density of macrophage lysosomes in order to substantiate our conclusions. To alter the density of lysosomes, immune guinea pigs were injected with Triton WR-1339 prior to macrophage harvest. Triton WR-1339 is a nondegradable detergent which has been shown to accumulate in lysosomes and consequently decrease their equilibrium density (22, 31). Conditions in Fig. 2B were identical to Fig. 2A except that immune guinea pigs were injected i.p. with Triton WR-1339 prior to macrophage harvest to decrease the density of the macrophage lysosomes. As shown, this treatment shifted both lysosomal marker enzymes to a lighter region of the gradient. In addition, the radiolabeled C. burnetii also shifted, thus supporting the conclusion that phagocytosis of phase I C. burnetii in the presence of immune serum by macrophages harvested from immune guinea pigs, results in sequestration of the rickettsiae in lysosomes.

Fig. 2C shows lysosomal enzyme and radioactivity profiles from immune macrophages infected in the presence of normal serum with

radiolabeled phase I C. burnetii. Once again, the radioactivity representing the phase I C. burnetii banded in the same region of the gradient as the macrophage lysosomal marker enzymes. However, in this case, when the lysosomes were shifted to a ligher density (Fig. 2D) with Triton WR-1339, the radioactivity did not shift, suggesting that in the presence of normal serum, phase I C. burnetii are not sequestered within the lysosomes of immune macrophages.

Similar experiments were also conducted with macrophages harvested from nonimmune guinea pigs. Equilibrium density profiles of lysosomal enzymes from nonimmune macrophages infected with radiolabeled phase I C. burnetii in the presence of immune serum are shown in Fig. 3A. The radioactivity banded in the same region of the gradient as the lysosomal enzymes. When these nonimmune guinea pigs were treated with Triton WR-1339 prior to macrophage harvest, the macrophage lysosomal enzymes and the radioactivity shifted to a lighter region of the gradient (Fig. 3B). This observation suggests that opsonized phase I C. burnetii also become sequestered within lysosomes of nonimmune macrophages.

The final set of experiments examined the intracellular localization of phase I C. burnetii in nonimmune macrophages in the presence of normal serum. The results of these experiments were not as easily interpreted as the previous ones. In the presence of normal serum, nonimmune macrophages phagocytized considerably less labeled C. burnetii (11, 18, 20). As a result, the amount of radioactivity in each fraction was only slightly above background. When macrophages from nonimmune guinea pigs were infected with phase I C. burnetii in the presence of normal serum, the radioactivity appeared to be in the same region of the gradient as the lysosomal enzymes (results not shown). After treatment with Triton WR-1339, there did not appear to be a shift of radioactivity. These

results suggest that in the presence of normal serum C. burnetii was not sequestered in the lysosomes of nonimmune macrophages.

DISCUSSION

Previous in vitro studies have shown that immune serum potentiates the destruction of C. burnetii (18-20) and other rickettsiae (12, 14) in macrophages. The mechanism by which this occurs, however, is not known.

We have presented data which show that immune serum potentiates phagosome-lysosome fusion in macrophages harvested from both nonimmune and immune guinea pigs. It is assumed that antibody is the constitutent of the immune serum which in some unexplained manner permits fusion of lysosomes with phagosomes resulting in subsequent degradation of C. burnetii within the phagolysosome.

The serum in these studies was heated for 30 min at 56°C, indicating that complement was not responsible for the observed results. It is possible, however, that the immune serum contained soluble products of activated lymphocytes. Since these have been shown to inhibit the intracellular growth of C. burnetii in cultures of nonimmune guinea pig peritoneal macrophages (9), their presence in the immune serum could be responsible for our results.

It should be noted that in vitro and in vivo results for both C. burnetii and Rickettsia akari do not correlate. In vitro, nonantibody-coated C. burnetii (18, 20) and R. akari (14) are both degraded by macrophages. In vivo, however, complete clearing of these infections does not occur in athymic mice even in the presence of antibody (14, 16), thus suggesting that functional T lymphocytes are required for complete clearance.

In support of this suggestion are the observations by Hinrichs and Jerrells (9) which show that immune lymphocytes or their soluble products are capable of inhibiting the intracellular growth of C. burnetii in nonimmune macrophages. In addition, it has been shown that macrophages activated in vitro by lymphokines were capable of suppressing the growth of Rickettsia tsutsugamushi in the absence of specific antibody (25). Other investigators have shown enhanced phagocytosis, bacteriostatic, or even bactericidal effects of macrophages in the presence of sensitized lymphocytes or soluble products of activated lymphocytes (6, 24, 26-28).

One possible explanation for the discrepancy in the in vitro and in vivo results may be that macrophages, recruited in vivo in response to sodium caseinate or mineral oil, may be activated and therefore more capable of killing antibody-coated rickettsiae (5). It is also possible that lymphocyte contamination of the macrophage cultures or their soluble products could be responsible for activating the macrophages. We used only adherent cells with 1% or less contamination by lymphocytes, but the role of sensitized lymphocytes or the soluble products of activated lymphocytes must be taken into account.

As discussed earlier (15) it appears that in vivo both humoral and cellular aspects of immunity are responsible for complete clearance of C. burnetii. We have attempted to provide insight on the humoral aspect, but cannot exclude possible contributions from the cellular components of immunity. We have shown, however, that the role of immune serum is to potentiate phagosome-lysosome fusion and permit subsequent degradation of C. burnetii in phagolysosomes. Other reports suggest that the ability of certain intracellular pathogens to prevent phagosome-lysosome fusion in macrophages may be important for their intracellular survival (2-4, 8, 10, 21, 32).

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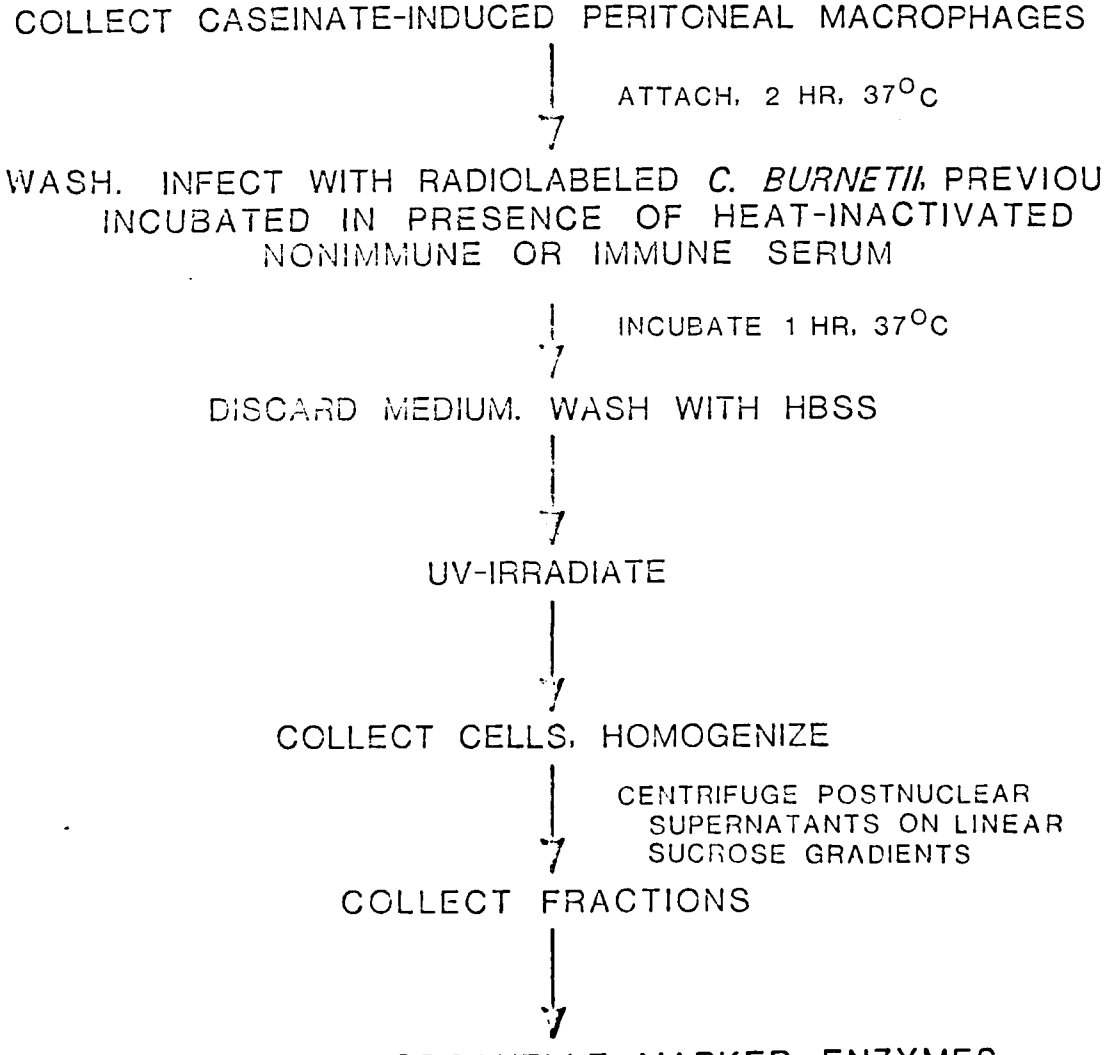
FIGURE LEGENDS

FIG. 1. Flow diagram of the experimental fractionation protocol C. burnetii-infected macrophages.

FIG. 2. Distribution profiles of two lysosomal marker enzymes (N-acetyl-β-glucosaminidase (GLCNAC) (--), α-galactosidase (GAL) (...) and radioactivity (TRACER) in sucrose gradients. A. From immune macrophages infection with radiolabeled phase I C. burnetii in the presence of immune serum. B. From Triton-loaded immune macrophages infected with radiolabeled phase I C. burnetii in the presence of immune serum. C. From immune macrophages infected with radiolabeled phase I C. burnetii in the presence of normal serum. D. From Triton-loaded immune macrophages infected with radiolabeled phase I C. burnetii in the presence of normal serum. Results are presented in the form of normalized and averaged frequency histograms. The density scale, divided into 15 normalized fractions of identical density increment, extends from 1.10 to 1.25. The frequency represents $\Delta Q/(\Sigma Q \cdot \Delta\rho)$, where ΔQ is the amount of constituent present within the section, and ΣQ the sum of the amounts found in all the subfractions. The surface area of each histogram bar then gives the fractional amount of constituent present within each normalized fraction. $\Delta\rho$ is equal to 0.0113 density units. Distribution profiles are flanked on either side by blocks arbitrarily constructed over the density spans 1.06 to 1.10 and 1.25 to 1.30 and refer to material recovered above and below the linear limits of the gradient. The total area of each histogram is then equal to 1. The numbers in the upper right hand corner of each figure represent the % recovery of enzyme activity.

FIG. 3. Same as Fig. 2 except (A) is from nonimmune macrophages infected with radiolabeled phase I C. burnetii in the presence of immune serum and (B) is from Triton-loaded nonimmune macrophages infected with radiolabeled phase I C. burnetii in the presence of immune serum.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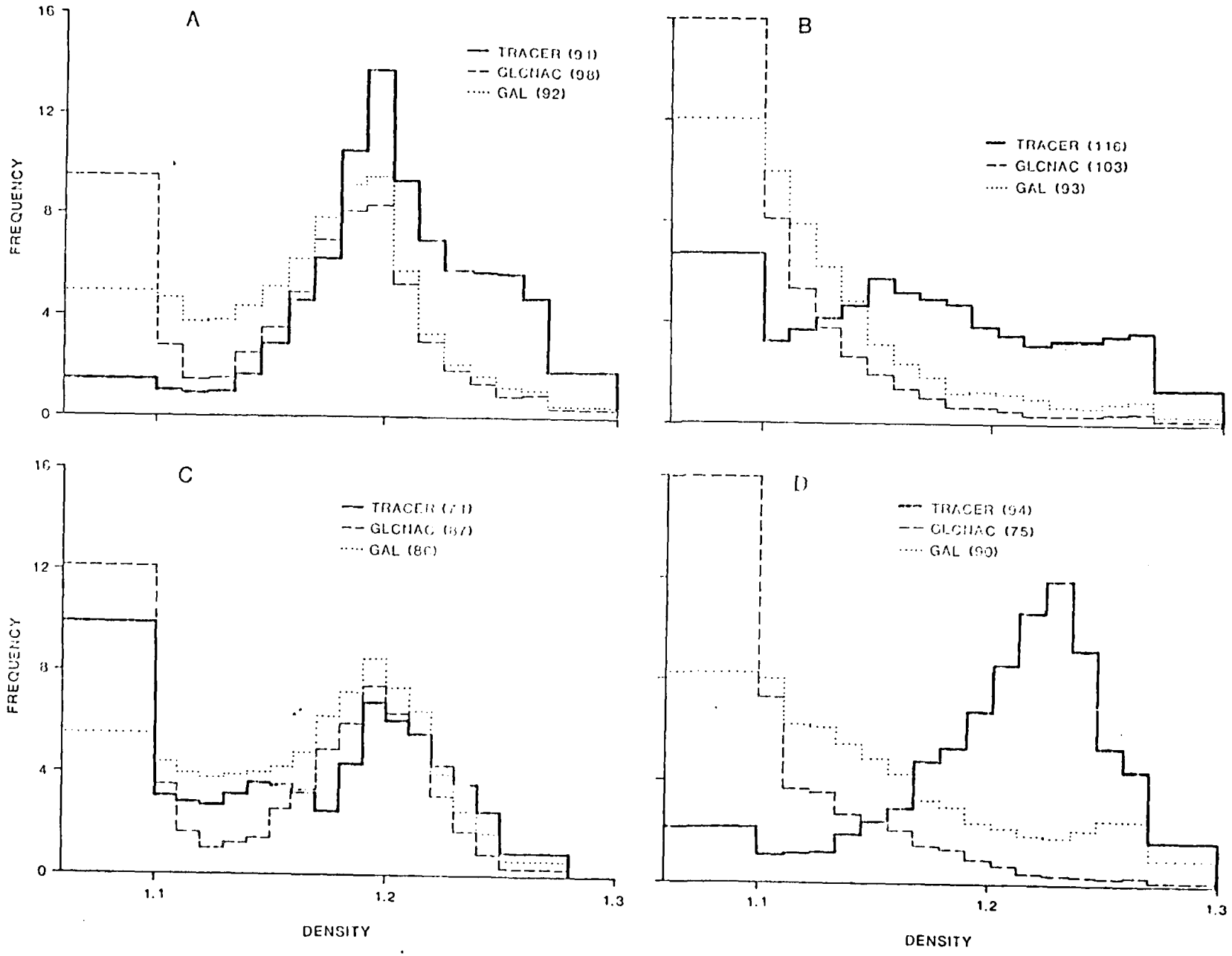
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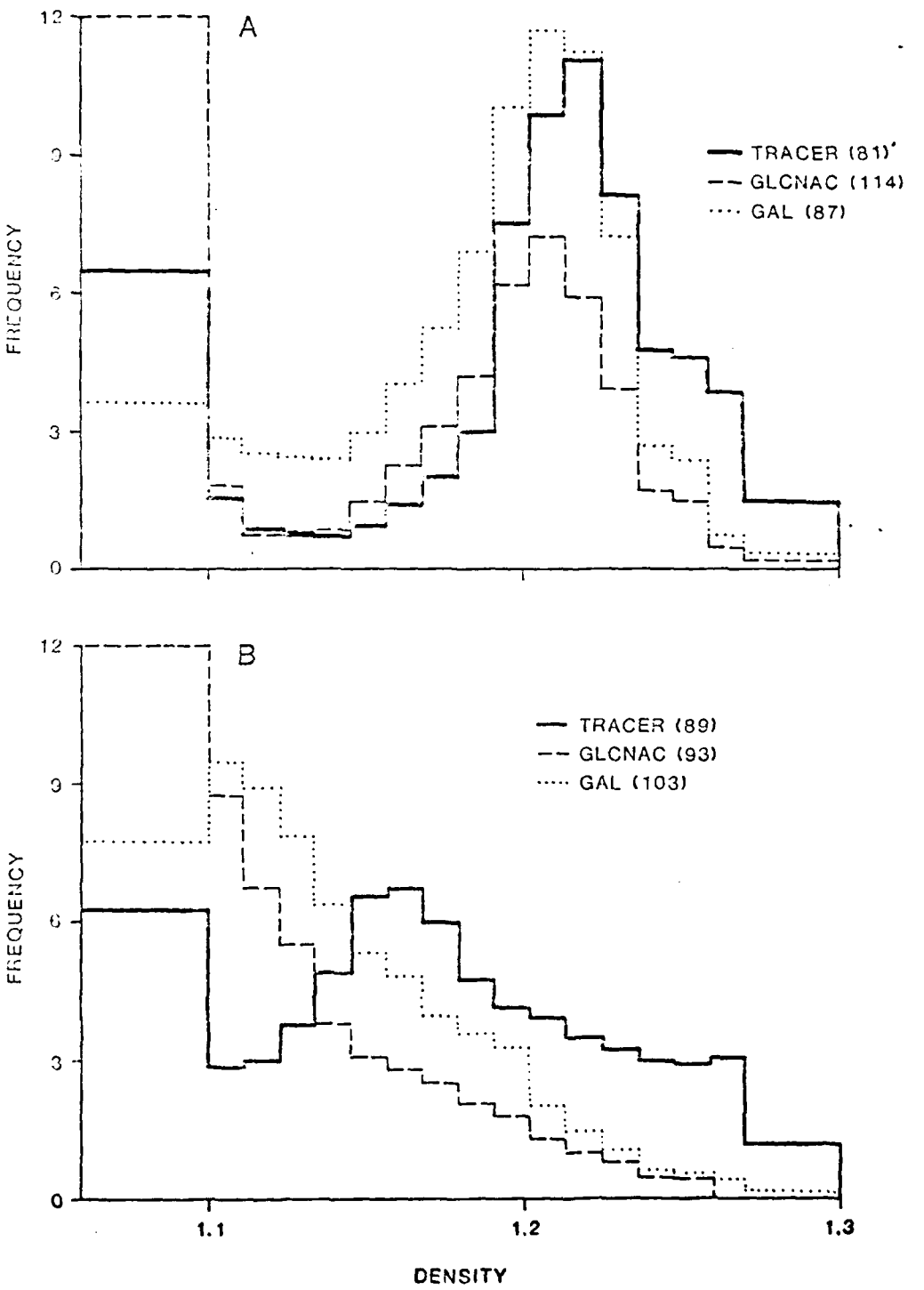
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